**B0172**

**Can clinical evaluation detect isolated one bundle or complete two bundles ACL rupture?**

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**Objectives**: Anterior cruciate ligament (ACL) is generally composed of anteromedial bundle (AMB) and posterolateral bundle (PLB). The function of AMB is not same as one of PLB. When they are injured, they should have different clinical features. At least, isolated one bundle ACL rupture should have different features compared to complete 2 bundles ACL rupture. The purpose of this study is to investigate the clinical features of a isolated one-bundle ACL rupture and a complete two-bundle ACL rupture. The hypothesis is that clinical evaluation, including physical examination and arthrometer measurements, can differentiate isolated from complete two-bundle ACL ruptures.
**Methods**: This is a prospective research (18 November 2003 to 09 August 2011). 525 ACL deficiencies were collected in this study. Two groups were confirmed by arthroscopy. 27 patients with isolated one bundle ACL rupture (SB group), including 11 AMB rupture (AMB group) and 16 PLB rupture (PLB group), and 498 patients with complete two bundles ACL rupture (DB group) were employed for the biomechanical test before and after anesthetic according to arthroscopy. Statistics was done between DB group and SB group in order to investigate whether clinical evaluation can detect isolated one bundle or complete two bundles ACL rupture. Statistics was done between AMB group and PLB group in order to investigate whether clinical evaluation can detect AM bundle or PL bundle rupture. The clinical evaluation include KT1000 (unit: mm), Lachman test (grade 4) and Pivot shift test (grade 5) before anesthetic and Lachman test (grade 4) and Pivot shift test (grade 5) after anesthetic. The data were analyzed using a unpaired *t* test, nonparametric test and discriminant analysis. The data of KT1000 was analyzed with un-paired *t* test. The data about Lachman and Pivot shift test was analyzed with Mann-Whitney test. It was assumed that there was statistical significance when $P < .05$. According to the variables with statistical significant result, two formulae were developed with discriminant analysis. All statistical data were calculated with the SPSS statistical software package (SPSS version 17.0, SPSS Chicago, IL, USA).
**Results**: In response to KT1000 side to side difference before anesthetic, between DB group ($3.4216 \pm 2.80607$mm) and SB group ($2.3077 \pm 1.82799$mm), there was significant differences between the two groups ($t$=2.002, $P$= 0.046). In response to Lachman test before anesthetic, there was significant differences between DB group and SB group (Z =-2.051, $P$= 0.04). In response to Lachman test after anesthetic, there was significant differences between DB group and SB group (Z =-3.913, $P$= 0.000). In response to Pivotshift test after anesthetic, there was significant differences between DB group and SB group (Z =-6.305, $P$= 0.000). In response to Lachman test before and after anesthetic, there was not significant differences between AMB group and PLB group (Z =-1.210, $P$= 0.226, before; Z =-1.719, $P$= 0.086, after). In response to Pivot shift test before and after anesthetic, there was not significant differences between AMB group and PLB group (Z =-0.397, $P$= 0.691, before; Z =-0.307, $P$= 0.79, after). According to discriminant analysis, two formulae were developed to diagnose isolated one bundle or complete 2 bundles ACL tears. Before anesthetic, Z>0.7783 indicates a two-bundle tear and Z< 0.7783 indicates an isolated bundle tear (False accept rate 0.049). After anesthetic, Z> 2.665895 indicates an isolated bundle tear and Z< 2.665895 indicates a two-bundle tear (False accept rate 0.053). In general, false accept rate <0.1 means that the formula is useful.
**Conclusion**: Clinical evaluation can differentiate an isolated one-bundle ACL rupture from a complete two-bundle ACL rupture. However, it cannot detect AM bundle rupture from PL bundle rupture.
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**B0174**

**Autophagy plays a protective role in tumor necrosis factor-α-induced apoptosis of bone marrow-derived mesenchymal stem cells**

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**Background**: Bone marrow-derived mesenchymal stem cells (BMSCs) are being broadly investigated for treating numerous inflammatory diseases. However, the low survival rate of BMSCs during the transplantation process has limited their application. Autophagy can maintain cellular homeostasis and protect cells against environmental stresses. Tumor necrosis factor-α (TNF-α) is an important inflammatory cytokine that can induce both autophagy and apoptosis of BMSCs. However, the actual role of autophagy in TNF-α-induced apoptosis of BMSCs remain poorly understood.
**Materials**: BMSCs were obtained from 10 healthy donors.
**Method**: In the current study, BMSCs were treated with TNF-α/cycloheximide (CHX), and cell death was examined by Cell Counting Kit-8, Hoechst 33342 staining, and flow cytometric analysis as well as by the level of caspase-3 and caspase-8. Meanwhile, autophagic flux was examined by analyzing the level of microtubule-associated protein light chain 3 B (LC3B)-II and SQSTEM1/p62 and by examining the amount of green fluorescent protein-LC3B by fluorescence microscopy. Then, the cell death and autophagic flux of BMSCs were examined after pre- and co-treatment with 3-methyladenine (3-MA, autophagy inhibitor) or rapamycin (Rap, autophagy activator) together with TNF-α/CHX. Moreover, BMSCs pre-treated with lentiviruses encoding short hairpin RNA (shRNA) of beclin-1 (BECN1) were treated with TNF-α/CHX, and then cell death and autophagic flux were detected. We showed that BMSCs treated with TNF-α/CHX presented dramatically elevated autophagic flux and cell death. Furthermore, we showed that 3-MA and shBECN1 treatment accelerated TNF-α/CHX-induced apoptosis but that Rap treatment ameliorated cell death.
**Results**: We showed that BMSCs treated with TNF-α/CHX dramatically elevated autophagic flux and cell death. Furthermore, we showed that pre- and co-treatment with 3-MA accelerated cell death but that Rap ameliorated TNF-α/CHX-induced apoptosis. Moreover, BMSCs pre-treated with shRNA of BECN1 were more vulnerable to TNF-α/CHX-induced apoptosis compared to the negative control.
**Discussion**: Our results demonstrate that autophagy protects BMSCs against TNF-α-induced apoptosis.
**Conclusion**: We demonstrate that enhancing autophagy has a protective effect against TNF-α/CHX-induced apoptosis, but inhibiting autophagy renders cells more susceptible to TNF-α/CHX-induced cell death. These results indicates the protective role of autophagy in BMSCs in the inflammatory environment, and enhancing autophagy may be a promising strategy to improve the survival capacity of BMSCs after transplanting to the inflammatory environment.
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**B0179**

**Effects of electrospun silk fibroin mats on tendon-bone healing**

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**Background**: Tendon-bone healing plays an important role after anterior cruciate ligament (ACL) reconstruction. The extracellular matrix (ECM) of organism is nanoscale, and by electrospinning, micro-/nanoscale scaffolds which have high porosity and surface area could be fabricated. It was demonstrated that compared with aligned electrospun mat, random electrospun mat could induce osteogenic differentiation of stem cells. Among the materials that can be electrospun, silk fibroin (SF) has drawn a great research interest because of excellent mechanical properties and biocompatibility. Based on the aforementioned information, we hypothesize that random electrospun SF mat wrapping could enhance tendon-bone healing of autograft.
**Material**: Cocoons of Bombyx mori silkworm and 32 New Zealand white rabbits (mean weight $2.8 \pm 0.5$ kg) were used in the study.
**Method**: The random electrospun SF mats were made from aqueous solution of regenerated SF. The New Zealand white rabbits were randomly divided into electrospun SF mat group and control group. After anesthesia, a 2-cm-long partial-thickness Achilles tendon of right lower limb was harvested and a bone tunnel was made in the proximal tibia of left lower limb at 60° angle to the vertical axis of the tibia by a 2.5 mm diameter kirschner wire. In electrospun SF mat group, the harvested Achilles tendon wrapped by electrospun SF mat was implanted into the bone tunnel, while in control group, the Achilles tendon without wrapping was implanted into the bone tunnel. After the operation, the rabbits were allowed to move freely in their separate cages without immobilization. The rabbits were sacrificed at 6 and 12 weeks postoperation for gross observation, histological observation and biomechanical tests.
**Results**: The electrospun SF mats were unidentifiable already at 6 weeks postoperation. Histological hematoxylin-eosin (HE) staining showed that at 6 weeks after surgery, the collagen fibers of the tendon-bone interface in electrospun SF mat group were organized while those in control group were chaotic. At 12 weeks after surgery, the tendon-bone interface in electrospun SF mat group became much narrower compared with that in control group, indicating new bone had grown into the transplanted Achilles tendon. At both 6 and 12 weeks after surgery, electrospun SF mats were not observed in electrospun SF mat group, indicating that the mats were absorbed already. Mechanical tests showed that at both 6 and 12 weeks after surgery, the failure loads of electrospun SF mat group were significantly higher than that of control group ($P < 0.05$).
**Discussion**: It was demonstrated that periosteum wrapping could enhance tendon-bone healing and decrease bone tunnel enlargement. However, the harvest of periosteum will lead to the injury of donor site and prolong the operation time. In this study, we demonstrated that the random electrospun SF mat could enhance tendon-bone healing. So the electrospun SF mat could substitute periosteum to promote tendon-bone healing after ACL reconstruction, showing great clinical application potential. However, we did not compare the effects of electrospun SF mats with periosteum on tendon-bone healing, so which one was better remained unclear. The electrospun mats are excellent vehicles to deliver drugs, so the electrospun SF mats with osteogenic drugs could be researched in future.
**Conclusion**: In this study, we demonstrated that the random electrospun SF mat wrapping autograft could enhance tendon-bone healing.
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**B0182**

**A morphologic and quantitative comparison of mechanoreceptors in the tibial remnants of the ruptured human anterior cruciate ligament**

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**Background**: Reconstruction of the ruptured anterior cruciate ligament (ACL) does not always result in the expected successful outcome. A satisfactory outcome may depend not only on the